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Dichelops melacanthus and *Euschistus heros* injury on maize: Basis for re-evaluating stink bug thresholds for IPM decisions

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ABSTRACT

This study compared injuries caused by different densities of adults and nymphs of *Dichelops melacanthus* and *Euschistus heros* to better assess the stink bug economic threshold (ET) for maize in integrated pest management. Thus, four different trials were conducted in Londrina, Brazil from 2017 to 2018 in the greenhouse and under field conditions. The first and second trial compared the degree of injuries caused by different adult densities of *D. melacanthus* and *E. heros* on maize, using artificial infestation. The third trial compared the capacity of adults and nymphs of both species to injury maize. The fourth trial evaluated different ETs under field conditions. The study demonstrated that the ET for stink bugs in maize should be three adults of *D. melacanthus* m^{-1} of row. A lower ET triggered a higher number of insecticide applications, but did not improve either yield or net income, as shown by economic analysis. Moreover, the potential of *E. heros* for damaging maize was shown to be low. The results show the control is not justified for densities up to 6 stink bugs m^{-1} of row, since yield was not reduced at these densities. Also, stink bug nymphs and adults might not produce the same injuries. Not only were adults of *D. melacanthus* more harmful to maize than nymphs of the same species but also than adults or nymphs of *E. heros*. Further research comparing the insect damage caused by different developmental stages is still needed in order to refine current ETs for future application.

1. Introduction

Maize (*Zea mays*) is one of the most widely grown cereal crop worldwide. It is sown on approximately 162,000 million hectares in about seventy countries (Dowswell et al., 2018). Among the pests that attack this crop, stink bugs were first recognized as a problem in 1985 (Townsend and Bessin, 2003). There are several different species of stink bugs that can feed on maize. Among these, the Neotropical Brown Stink Bug, *Euschistus heros* (Fabricius 1798) (Hemiptera: Pentatomidae) is the most frequent in Brazil (>90% of the stink bug complex) (Corrêa-Ferreira, and Azevedo, 2002; Bueno et al., 2015). However, more recently, the Green-Belly Stink Bug, *Dichelops melacanthus* (Dallas 1851) (Hemiptera: Pentatomidae), has become more abundant and significant as a key pest in maize production (Duarte et al., 2015; Bueno et al., 2015). The overall increase of the importance of stink bugs to maize production is mostly a consequence of the adopted system of two crops per year: soybean in summer (first crop) immediately followed by maize in autumn (second crop) (Chiesa et al., 2016). This intensive field use provides a continuous food supply for insects throughout the year, known as the 'green bridge', which favors stink bug outbreaks (Smaniotto and Panizzi, 2015). This is due to large numbers of stink bugs that remain in the area after soybean harvest that attack recently germinated maize seedlings (Silva et al., 2013).

Currently, the most commonly applied pest control strategy is the spraying of chemical insecticides (Furlan and Kreutzweiser, 2015; Dowswell et al., 2018). However, for crop management to be sustainable, it is crucial to adopt integrated pest management (IPM) strategies. IPM is based on the premise that certain levels of plant pests are tolerable without reducing economic production (Higley and Peterson, 1996). In this context, the lowest density of a pest population that will cause economic damage to plants was defined as the economic injury level (EIL) (Stern et al., 1959). To avoid yield loss by reaching the EIL, the economic threshold (ET) was introduced. It defines the population density at which control measures should be initiated to prevent a pest population from exceeding the EIL (Pedigo et al., 1986; Prokopy and Kogan, 2003). Still, information regarding the ETs for stink bugs in

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maize is scarce. Moreover, the recommended ET depends on the situation and thus varies from 0.18 (Hooks, 2011) to 0.5 stink bugs m^{-1} of row (Chiaradia et al., 2016). While those ETs were only estimated for adults of *D. melacanthus*, damage might differ between stink bug species and developmental stages. The quantification of these differences is relevant to recommendations for IPM and needs further investigation.

Stink bugs feed on plant fluids by inserting their needlelike mouthparts into stems, leaves or seeds, thereby causing injuries that can lead to plant death depending on the intensity and stage of plant development in which the attack occurs (Chocorosqui and Panizzi, 2004). During ingestion, stink bugs inject substances into the plant that facilitate digestion and sap removal. The feeding behavior might vary between different stink bug species (Depieri and Panizzi, 2011), as well as between adults and nymphs of the same species, which might lead to different plant tolerance and species-specific and insect life stage-specific ETs. Dead seedlings, stunted plants, or tillering are symptoms of injury prevalent in maize fields (Townsend and Bessin, 2003; Queiroz et al., 2017; Valicente, 2015), but it is unknown to which extent the mechanical and chemical damage to the growing point of plants by different stink bugs and their developmental stages are responsible for these symptoms.

Although *D. melacanthus* is considered the most dangerous pentatomid to maize in Brazil, *E. heros* can also be frequently found feeding on maize plants. Thus, the presence of not only *D. melacanthus* but also of *E. heros* has encouraged growers to spray insecticides in maize fields to control pest outbreaks. It is therefore of great theoretical and practical interest to understand the different injury potentials of *D. melacanthus* and *E. heros* adults and nymphs (Torres et al., 2013), allowing a more precise ET development and, therefore, improved stink bug management in the field. Thus, in order to better assess the stink bug economic threshold for maize in IPM decisions, the aim of this study was 1) to examine injuries caused by different densities of *D. melacanthus* and *E. heros* adults, and 2) to compare the injury potential between nymphs and adults of each species.

2. Materials and methods

Four different trials were carried out in Londrina, Parana, Brazil from 2017 to 2018 in the greenhouse and under field conditions to study the potential damage of stink bugs to maize as briefly described in the following.

2.1. Laboratory rearing of D. melacanthus and E. heros

Both *D. melacanthus* and *E. heros* used in the artificial infestation trials (trials 1, 2, and 3) were obtained from a mass-reared colony kept in plastic cages (25 x 20 × 20 cm) at 25 ± 2 °C, 60 ± 20% relative humidity, and 12:12 h L:D photoperiod at Embrapa Soja (Londrina, Parana, Brazil). The insects were fed *ad libitum* with a mixture of fresh green bean pods *Phaseolus vulgaris* (L.), dry soybean seeds *Glycine max* L.; raw shelled peanuts *Arachis hypogaea* L., and sunflower seeds *Helianthus annuus* L. as described by Silva et al. (2008, 2011).

2.2. Comparison of injury rates of different densities of E. heros and D. melacanthus adults on maize plants (trials 1 and 2)

Two independent trials (one for each species) were carried out under field conditions in a randomized block design with seven treatments (zero, one, two, three, four, five and six stink bugs of each species per cage) and five replicates (cage of 1 m × 1 m with 6 plants) at Embrapa Soja Experimental Farm S 23° 11′ 11.7"; WO 51° 10′ 46.1″ from March to September 2017. The maize hybrid used was BM 709 PRO2, which expresses the Bt traits Cry1A.105 and Cry2Ab2. Each cage had a size of 1 m^3 (1m × 1m x 1m) and consisted of iron bars covered with nylon screen, including a door fitted with a Velcro stripe which allowed its opening and closing for evaluation and maintenance of the experiment.

One day before sowing, 234 kg ha^{-1} of N–P–K (08-28-16), and 18 days after sowing, 150 kg ha^{-1} of urea were applied as soil fertilizers. Two days after plant emergence [at Emergence (VE) stage of plant development] cages were assembled and infested with stink bugs according to each treatment. Every other day the cages were monitored, and dead or escaped stink bugs were replaced. Infestation was maintained until 28 days after the first day of infestation (DAI). After this period, cages were removed and plants were sprayed with insecticides and kept free of pests until harvest. Evaluations were carried out throughout the development of the plant, pre-harvesting and post-harvesting.

Throughout plant development, evaluations were carried out on four dates (7, 14, 21 and 28 DAI). The evaluated parameters were a) plant injury rating (Roza-Gomes et al., 2011) where: 0 = no damage, 1 = leaves with small punctuations, plant with no size reduction, 2 = injured whorl (partially twisted), plant with size reduction, 3 = twisted whorl or "suckering" plants (plants with tillers from the base), and 4 = dead whorl; b) plant height (cm); and c) number of expanded leaves $plant^{-1}$. At pre-harvesting, the measured parameters were: a) main cob height (cm) and b) number of cobs $plant^{-1}$. Finally, at post-harvesting, the measured parameters were: a) main cob length (cm); b) grain rows per cob; c) weight of 1000 grains (grams); and d) grain yield at 13% humidity (kg ha^{-1}). Grain moisture was measured using the G800 moisture meter (Gehaka Agri, São Paulo-SP, Brazil) before correcting the yield for 13% humidity.

2.3. Comparison of injury by adults and nymphs of E. heros and D. melacanthus on maize (trial 3)

To compare injuries caused by adults and third-instar nymphs of *D. melacanthus* and *E. heros*, a trial was carried out in the greenhouse in a completely randomized design with five treatments (1 adult of *D. melacanthus*, 1 nymph of *D. melacanthus*, 1 adult of *E. heros*, 1 nymph of *E. heros*, and control with no insects) and ten replicates (cage of 35 × 50 cm with 1 plant, infested with a single insect) at Embrapa Soja, Londrina, Parana, Brazil, from April to May 2018. Plastic pots with a capacity of 5 L were used for planting. Stink bugs were introduced to the cages two days after plant emergence (BM 709 PRO2 hybrid at the VE stage of plant development), and kept for ten days. Every two days the cages were monitored, replacing dead stink bugs or nymphs that had developed to the fourth instar. Thus, infestation was maintained with either adults or 3rd instar nymphs during the entire trial.

After a 10-day infestation period, cages and insects were removed. Evaluations were performed at two time points: the first one after the stink bugs were removed (day zero after insect removal; 0 DAIR), and the second seven days thereafter (7 DAIR). At both days of evaluation (0 and 7 DAIR), the evaluated parameters were plant injury rating (Roza-Gomes et al., 2011), plant height (cm) and number of expanded leaves. At 7 DAIR, in order to evaluate shoot dry matter (grams), root dry matter (grams) and total dry matter (shoot + root dry matter), plants were cut at the base of the substrate, separating the aerial part (shoot) from the root. The roots were washed with pure water. All parts were packed in individual paper bags and taken to a 60 °C oven with forced air circulation for five days, until reaching constant weight.

2.4. Field evaluation of different economic thresholds (ET) for stink bugs in maize (trial 4)

The trial was carried out under field conditions in a randomized block design with four treatments (insecticide applied weekly, no insecticide, insecticide applied with one stink bug m^{-1} of row, and insecticide applied with two stink bugs m^{-1} of row) and four replicates (test area of 18 m width and 24 m length). One day before sowing, the soil was fertilized with 259 kg ha^{-1} of N–P–K 08-28-16 + 0.3% of Zn. The trial field was sown on March 2nd, 2018 with the cultivar ' BM 709 PRO2 ′ at 6 seeds per meter and 90 cm between rows. Third five days after sowing, 100 kg ha^{-1} of urea was applied to the soil as additional plant fertilizer.

As Bt maize was used for the trials, insecticides for caterpillars were not needed. Herbicides (glyphosate 1440 g.a.i. ha^{-1} Roundup 3L ha^{-1}) and fungicides (azoxystrobin + cyproconazol 93.33 g.a.i. ha^{-1}; Priori Xtra® 300 mL ha^{-1}) were applied in all treatments (including the control – no insecticide) to isolate the effect of stink bug infestation in the experiment. Throughout the duration of the experiment (March to August), herbicides were applied once (2 weeks after maize emergence). Fungicides were applied once at the beginning of the reproductive stage.

The insecticide used was thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g.a.i. ha^{-1} (Engeo Pleno® 250 mL ha^{-1}), applied whenever the economic threshold (ET) of a treatment had been reached. To manage stink bugs, different thresholds were used to initiate insecticide application (1 stink bug m^{-1} of row and 2 stink bugs m^{-1} of row). There was also a third treatment without any applications (control) and a fourth treatment with insecticides applied weekly (no insect injury). All pesticides (herbicides, fungicides, and insecticides) were applied with the sprayer model "Advance 2000 AM18 Vortex" (Jacto, Pompéia – São Paulo, Brazil) adjusted to a spray volume of 150 L ha^{-1} using a tip model AXI-110-02. Spraying was carried out under appropriate environmental conditions (winds below 6 km h^{-1}, relative humidity above 50%, and a maximum temperature of 25 °C).

Insect samples were collected weekly, starting from the VE stage until 28 days after insect infestation (which was 30 days after plant emergence and at the V7 stage of plant development, i.e. with seven expanded leaves). For this, 2 m of each row (12 plants) were visually inspected (including a distance of 20 cm on both sides). In each replicate, four random samples were taken, counting stink bugs bigger than 0.5 cm (corresponding to adults and to nymphs from 3rd to 5th instars). All individuals were identified to species. In addition, at each sample point, 10 plants were also evaluated according to the plant injury rating (Roza-Gomes et al., 2011) as previously described for trials 1, 2 and 3.

At pre-harvest, flag leaf height (meter), main cob height (meter), and number of cobs per 10 m were measured at two points within a 5 m distance. Subsequently plants were harvested at these points. Samples were then threshed individually and evaluated. Weight and moisture content of each sample were recorded, and corrected for yield (adjusted to 13% seed moisture). Grain moisture was measured using the G800 moisture meter (Gehaka Agri, São Paulo, Brazil) and corrected for productivity at 13% relative humidity.

2.5. Data analysis

An economic analysis was carried out following the methodology of Corrêa-Ferreira et al. (2010) and applying maize prices of US$ 160/maize tonne in 2017 and an average of US$ 39.25/hectare/insecticide application (US$ 14.25/hectare/application + spraying cost of US$ 25.0/hectare/application) (FAOstat, 2019). Net income was considered as total yield minus the costs of stink bug insecticide used for each treatment converted to kilograms of maize.

Net income as well as all collected data were analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972), and, if necessary, transformed prior to performing ANOVA. The treatment means were then compared by Tukey test at the 5% probability level (SAS Institute, 2001).

3. Results

Euschistus heros adults had no impact on any of the evaluated parameters (Figs. 1–4, trials 1 and 2) at a density of up to 6 adults m^{-1} of row (6 plants). There was no difference between *E. heros* infested plants and non-infested plants of any of the evaluated parameters or on any of the evaluation days: plant injury rating (Fig. 1), plant height (Fig. 2),

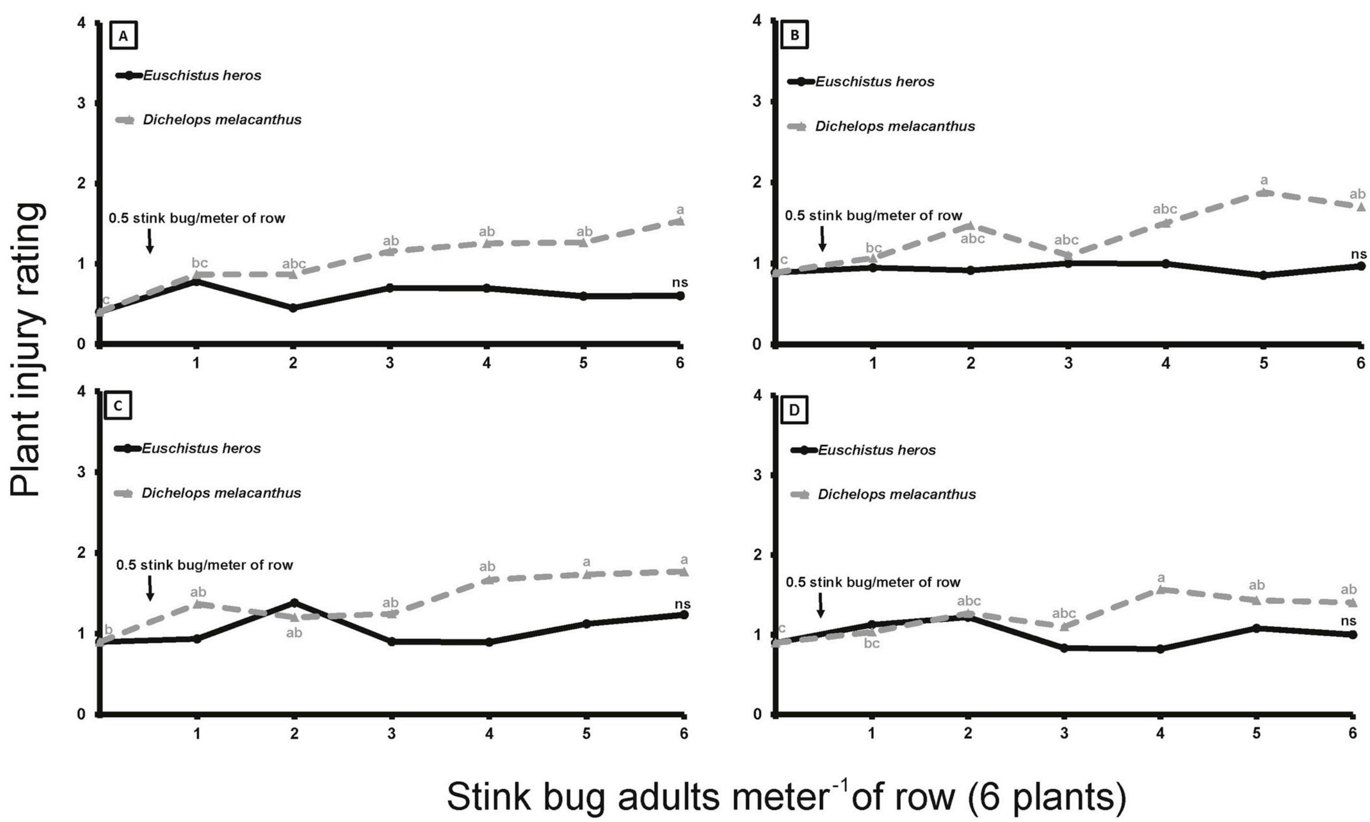

Fig. 1. Plant injury rating of maize (BM 709 PRO2) resulting from different stink bug densities (adults of *Euschistus heros* and *Dichelops melacanthus*) 7 (**A**), 14 (**B**), 21 (**C**) and 28 (**D**) days after insect infestation (trials 1 and 2). Means followed by different letters, for each species and evaluation date statistically differed according to the Tukey test ($p \leq 0.05$). [ns]ANOVA not significant. Trials carried out in Londrina, Parana, Brazil on2017.

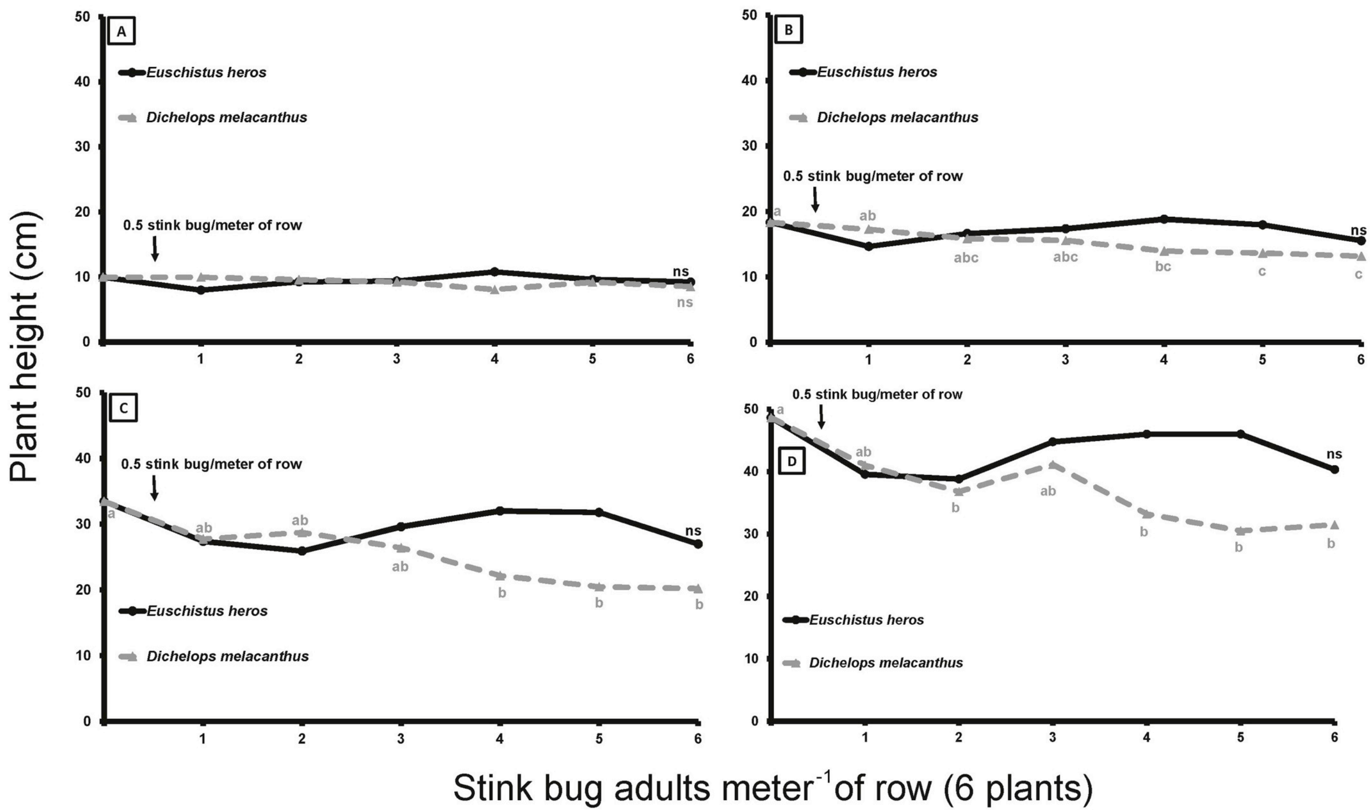

Fig. 2. Plant height (maize BM 709 PRO2) at different stink bug densities (adults of *Euschistus heros* and *Dichelops melacanthus*) 7 (**A**), 14 (**B**), 21 (**C**) and 28 (**D**) days after infestation (trials 1 and 2). Means followed by different letters, in each species and evaluation date statistically differed according to the Tukey test ($p \leq 0.05$). [ns]ANOVA not significant. Trials carried out in Londrina, Parana, Brazil on 2017.

number of expanded leaves per plant (Fig. 3), main cob height (Fig. 4A), number of cobs per plant (Fig. 4B), main cob length (Fig. 4C) and number of grain rows per cob (Fig. 4D). Even though there were differences among *E. heros* treatments regarding the weight of 1000 grains ($p = 0.0007$, F = 6.06, df = 32; Fig. 4E), infested treatments did not differ from the control. There was no difference in yield among *E. heros* treatments ($p = 0.1685$, F = 1.68, df = 34; Fig. 4F).

In contrast, *D. melacanthus* infested plants differed from non-infested plants regarding most of the evaluated parameters (Figs. 1–4). At the early developmental stages of plants (7 DAI), insects at densities of 3, 4, 5 and 6 *D. melacanthus* adults per meter of row (6 plants) resulted in a plant injury rating that differed from non-infested plants ($p = 0.0001$, F = 7.74, df = 33; Fig. 1A). These differences only occurred at levels of 5 and 6 *D. melacanthus* adults per meter of row with older plants, 14 ($p = 0.0043$, F = 4.53, df = 31; Figs. 1B) and 21 DAI ($p = 0.0196$, F = 3.17, df = 34; Fig. 1C). At 28 DAI ($p = 0.0017$, F = 5.08, df = 34; Figs. 1D), 4 and 5 and 6 adults of *D. melacanthus* per meter of row (6 plants) also resulted in a plant injury rating that differed from non-infested plants.

Similarly, plant height (cm) at 14 DAI (Figure 2B), 21 DAI (Figure 2C) and 28 DAI (Fig. 2D), main cob height (Fig. 4A) and yield (Fig. 4F) differed between non-infested plants and infested plants at levels of 4, 5 and 6 adults of *D. melacanthus* per meter of row (6 plants). No differences between *D. melacanthus* treatments were observed for plant height at early infestation (7 DAI) (Fig. 2 A), number of expanded leaves per plant (Fig. 3), main cob length (Fig. 4C) number of grain rows per cob (Fig. 4D) and weight of 1000 grains (Fig. 4E). Regarding the number of cobs per plant, differences were only noticed for densities of 5 and 6 adults of *D. melacanthus* per meter of row compared with non-infested plants ($p = 0.0045$, F = 4.29, df = 34; Fig. 4B).

The comparison of injury capacities of adults and 3rd instar nymphs of *D. melacanthus* and *E. heros* (trial 3) clearly indicated the highest injury capacity for adults of *D. melacanthus* (Table 1). After 10 days of insect infestation or 0 days after insect removal, plant injury rating was higher for adults of *D. melacanthus* (2.6) compared with injuries recorded for 3rd instar nymphs of *D. melacanthus* (0.9), as well as adults (1.4) and 3rd instar nymphs (0.8) of *E. heros* ($p < 0.0001$, F = 25.3, df = 49). After 10 days of infestation, stink bugs were removed from plants, which were allowed to recover from injuries. Even seven days after insect removal (DAIR), plant injury triggered by adults of *D. melacanthus* was higher than by nymphs of both species (plant injury rate, $p < 0.0001$, F = 20.6, df = 49). Higher injury caused by adults of *D. melacanthus* compared with 3rd instar nymphs as well as nymphs and adults of *E. heros* was also reflected in lower plant height (4.8 cm) ($p < 0.0001$, F = 10.4, df = 49), a smaller number of expanded leaves (3.0 leaves) ($p = 0.0031$, F = 4.7, df = 48), smaller shoots (0.9 g) ($p < 0.0001$, F = 11.4, df = 48), roots (0.4 g) ($p < 0.0001$, F = 9.3, df = 47), and total dry matter (1.3 g) ($p < 0.0001$, F = 12.8, df = 48) (Table 1).

In the field trial (trial 4), a higher number of stink bugs was recorded in treatments with reduced ET (1 stink bug m^{-1} of row) or with insecticide spraying on a weekly basis (Fig. 5A). In addition, a significantly higher plant injury was recorded for untreated plots three weeks after plant emergence (Fig. 5B). In spite of these results, there was no difference in yield among treatments ($p = 0.5981$, F = 0.66, df = 15, Table 2). In addition, the net income analysis indicates that a higher use of insecticides is disadvantageous. Natural infestation up to 2 stink bugs m^{-1} of row did not impact any of the evaluated productivity parameters. There was no difference between treatments (weekly spray, ET = 1 stink bug m^{-1} of row, 2 stink bugs m^{-1} of row or untreated) for grain yield ($kg.ha^{-1}$), weight of 1000 grains (grams) ($p = 0.2218$, F = 1.77, df = 15), number of cobs per 10 m ($p = 0.4733$, F = 0.91, df = 15), flag leaf height (meter) ($p = 0.1048$, F = 2.75, df = 15) and main cob height ($p = 0.3807$, F = 1.15, df = 15) (Table 2).

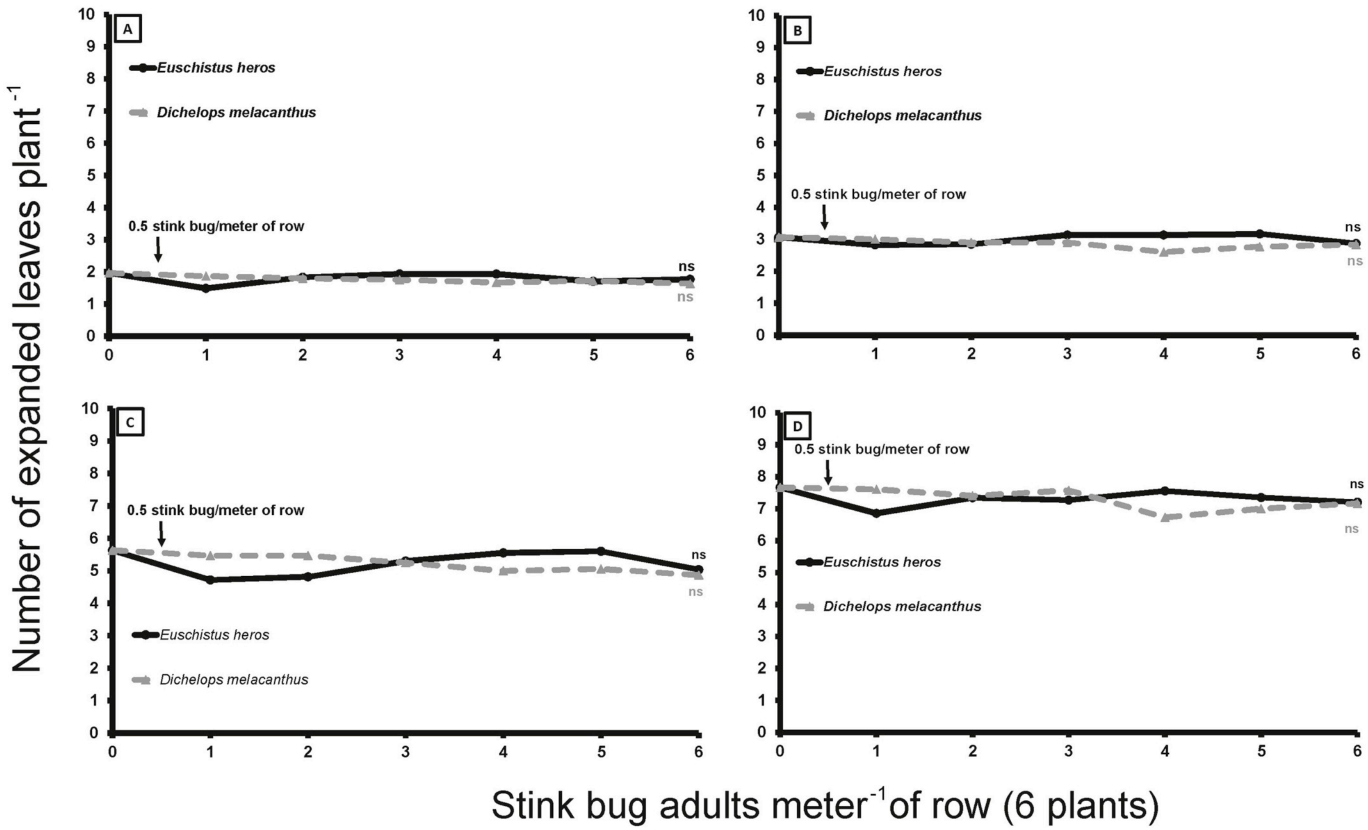

Fig. 3. Number of expanded leaves per plant (maize BM 709 PRO2) at different stink bug densities (adults of *Euschistus heros* and *Dichelops melacanthus*) 7 (**A**), 14 (**B**), 21 (**C**) and 28 (**D**) days after infestation (trials 1 and 2). Data of *D. melacanthus* at 7 DAI was analyzed after $\sqrt{X}$ transformation. ^{ns}ANOVA not significant. Trials carried out in Londrina, Parana, Brazil, on2017.

4. Discussion

In this study, we demonstrated that the ET for stink bugs in maize should be higher than suggested by most papers previously published on the subject. In addition, the collected data indicate that there are great differences in injury potential between nymphs and adults as well as between different stink bug species, a fact that should be examined closely in future research in order to allow ETs to be refined. Not only were adults of *D. melacanthus* more harmful to maize than nymphs of the same species but also than both adults and nymphs of *E. heros*. Higher damage potential of *D. melacanthus* compared with *E. heros* had previously been reported in the literature (Roza-Gomes et al., 2011; Copatti and Oliveira, 2011; Torres et al., 2013). However, to our best knowledge, this is the first study to report a higher injury capacity of *D. melacanthus* adults compared with nymphs of the same species. It is important to point out that the trial was carried out in a greenhouse and only for a period of 17 days. Greenhouse cultivation is not ideal for maize, a plant that is extremely affected by photoperiod and other variables that might differ from field conditions inside greenhouses. It is therefore necessary in the future to carry out trials in the field in order to validate the results herein reported.

Stink bug injury to plant tissues depends on penetration frequency of the insect stylet, length of feeding period and composition of salivary secretions, which may be toxic to the plant (Lucini and Panizzi, 2017, 2018). Food activity increases according to stink bug development, which could be an explanation for the differences between nymph and adult injury potential observed in this study for *D. melacanthus*. Another reason could be the difference in rostrum length observed between these developmental stages (Depieri and Panizzi, 2010). Undoubtedly, the interaction of both factors contributes to the higher injury capacity of *D. melacanthus* adults.

However, rostrum length cannot account for the difference in injury potential between adults of *E. heros* and *D. melacanthus* since it is similar in both species. Also, there was no difference in injury potential observed between adults and nymphs of *E. heros,* despite their different rostrum lengths (Depieri and Panizzi, 2010). A plausible explanation could be a different feeding behavior between stink bugs species. The feeding behavior of piercing-sucking insects is complex and highly sophisticated with all activities related to feeding occurring inside the plant tissue, causing difficulties for direct observation and quantification of feeding, which helps to explain the lack of information regarding this subject. The recently developed tool called Electropenetrography (EPG) (previously known as electrical penetration graph) provides an important technological advance for studying the interactions between stink bugs and their host plants, and could be applied to elucidate the observed differences in injury potential between species. As it is of theoretical and practical significance for the refinement of ETs (Lucini and Panizzi, 2018), this subject should be the focus of future research due to its importance for maize stink bug management.

Currently, the control of stink bugs in maize is primarily through insecticide applications. This reinforces the importance of appropriate pest management, with pest population monitoring and adoption of insecticides only when the ET is reached. Among the few studies that have evaluated the impact of stink bug infestation on maize, Chiaradia et al. (2016) observed that an ET of 0.5 stink bugs m^{-1} of row did not affect yield. Economic threshold (ET) values vary among publications, but are low in general, suggesting that a high amount of insecticide is required during the maize season. In the USA, the ET for stink bug feeding on maize less than 24 inches tall is 10% or more infested plants. This ET should be reduced to 3–5% of injured plants when stink bugs are still present (Hooks, 2011). In Brazil, the currently recommended ET for controlling stink bugs in maize varies from 0.27 (Bridi et al., 2016) to 0.8

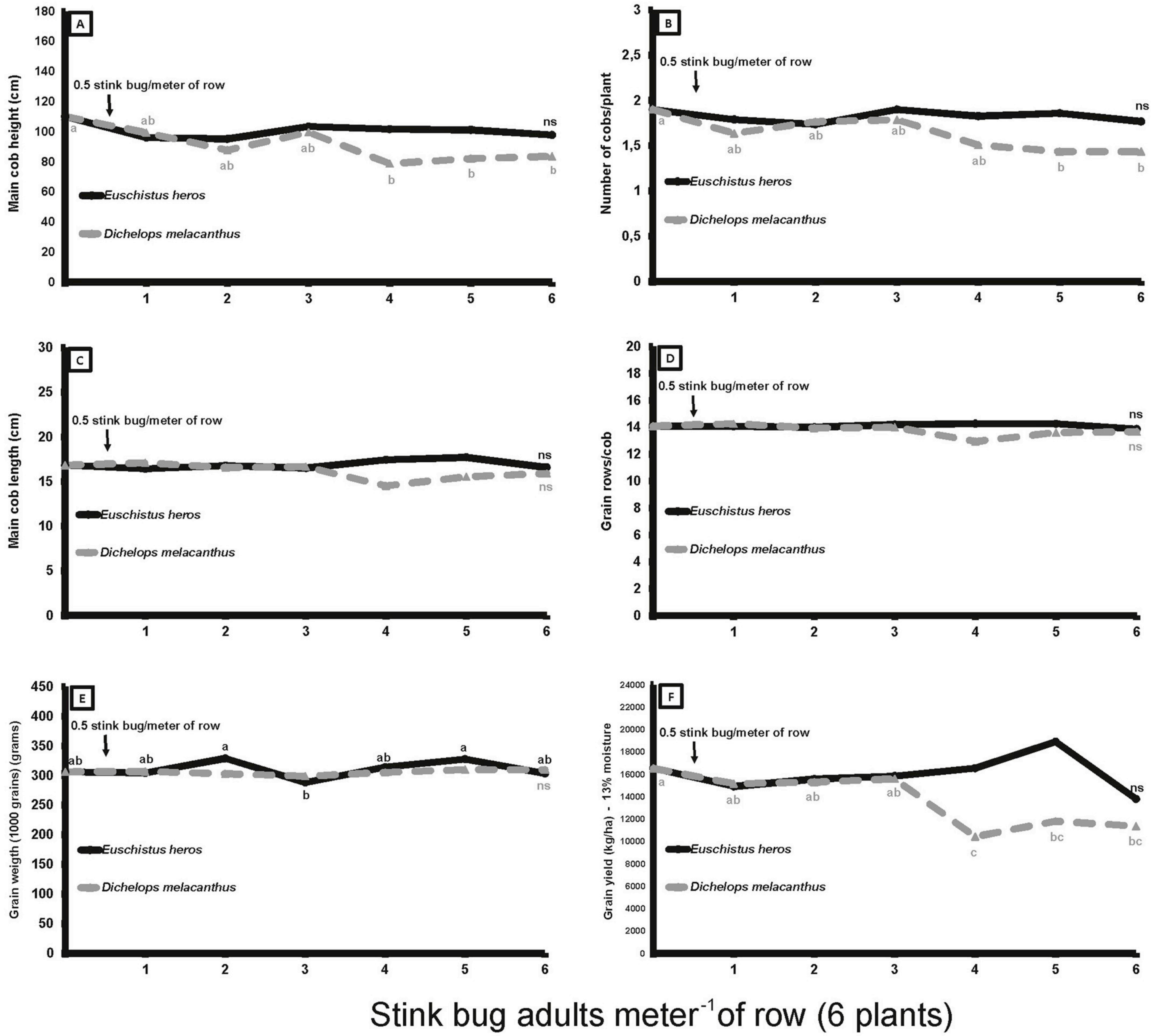

Fig. 4. Pre-harvesting and post-harvesting evaluated parameters (maize BM 709 PRO2) at different stink bug densities (adults of *Euschistus heros* and *Dichelops melacanthus*) 7 (**A**), 14 (**B**), 21 (**C**) and 28 (**D**) days after infestation (trials 1 and 2). Means followed by different letters, in each species and evaluation date statistically differed according to the Tukey test ($p \leq 0.05$). nsANOVA not significant. Trials carried out in Londrina, Parana, Brazil on 2017.

insects m^{-2} (Duarte et al., 2015). Alternatively, it is stated as insects per meter (0.5 stink bugs m^{-1} of row) (Chiaradia et al., 2016). In the present study, an ET of 2 stink bugs m^{-1} of row (6 plants) did not result in any yield reduction and, therefore may safely be adopted. In the natural infestation field trial (trial 4), an ET higher than 2 stink bugs m^{-1} of row could not be evaluated due to the lack of pest outbreaks that reached higher infestation levels. However, in artificially infested trials (trials 1 and 2), with as many as 3 adults of *D. melacanthus* m^{-1} of row, the maize yield was not reduced compared with the control. These results suggest that at least for the studied maize cultivar (BM 709 PRO2), an ET of 3 adults of *D. melacanthus* m^{-1} of row (6 plants) will not result in any yield reduction. Furthermore, adults of *D. melacanthus* triggered higher injury than its 3rd instar nymphs, a result that might require an ET refinement taking these differences into account.

Improper management of pests, particularly stink bugs, has been a great challenge in maize production. Currently, the indiscriminate use of insecticides associated with the lower ET adopted for stink bugs in maize is causing an environmental imbalance, and allows for selection of stink bugs resistant to the used insecticide and the resurgence of different pest species (Bueno et al., 2013; Panizzi, 2013). In our study, in addition to similar yields among the stink bug management thresholds, the adoption of a higher ET led to a higher net income than the stricter control with reduced ET levels. These results show that although a lower ET resulted in lower plant injury rating and lower insect densities achieved by higher insecticide applications, this practice did not increase the yield and should therefore not be adopted. Unnecessary insecticide spraying, which, apart from not providing any economic benefits, can negatively impact the agroecosystem, reducing the diversity of beneficial organisms in the area and increasing pest abundance (Lundgren and Fausti, 2015), and can be avoided by using higher ET levels.

The increasing importance of this stink bug in Brazil can be attributed to a combination of factors: (1) selection of stink bug populations resistant to the main insecticides used, (2) lack of market availability of insecticides with different action mechanisms, (3) poor application

Table 1

Plant parameters of maize (BM 709 PRO2) infested after 2 days of plant emergence for a total of 10 days in the greenhouse with 1 stink bug (adult or nymph) of *D. melacanthus* or *E. heros* per plant. Measurements were taken on the days of insect removal from cages and 7 days thereafter (trial 3). Trial carried out in Londrina, Paraná, Brazil on 2018.

Parameter	DAIR[a]	Treatment					Statistics			
		Untreated	*Dichelops melacanthus*		*Euschistus heros*					
			Adult	3rd instar	Adult	3rd instar	CV	p	F	df
Plant injury rating[b]	0[c]	0.0 ± 0.0 c	2.6 ± 0.3 a	0.9 ± 0.2 b	1.4 ± 0.2 b	0.8 ± 0.1 b	19.5	<0.0001	25.3	49
	7[c]	0.0 ± 0.0 c	1.6 ± 0.2 a	0.8 ± 0.2 b	1.1 ± 0.1 ab	0.8 ± 0.1 b	16.9	<0.0001	20.6	49
Plant height (cm)	0	10.8 ± 0.6 a	4.8 ± 1.0 c	7.8 ± 0.8 b	9.0 ± 0.6 ab	9.0 ± 0.4 ab	26.3	<0.0001	10.4	49
	7	18.8 ± 1.1 a	12.2 ± 1.1 b	16.9 ± 0.9 a	16.3 ± 1.0 a	18.4 ± 0.7 a	18.7	0.0001	7.3	49
Number of expanded leaves	0	3.9 ± 0.1 a	3.0 ± 0.2 b	3.7 ± 0.2 a	3.5 ± 0.2 ab	3.6 ± 0.2 ab	13.4	0.0031	4.7	48
	7	6.1 ± 0.1[ns]	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.7	0.3980	1.0	49
Shoot dry matter (g)	7[d]	2.6 ± 0.2 a	0.9 ± 0.1 c	2.0 ± 0.2 ab	1.9 ± 0.2 b	2.3 ± 0.2 ab	31.7	<0.0001	11.4	48
Root dry matter (g)	7[d]	1.3 ± 0.1 a	0.4 ± 0.1 c	0.8 ± 0.1 bc	0.8 ± 0.1 ab	1.2 ± 0.2 ab	20.8	<0.0001	9.3	47
Total dry matter (g)	7[d]	3.9 ± 0.3 a	1.3 ± 0.2 c	2.8 ± 0.3 ab	2.7 ± 0.4 b	3.5 ± 0.3 ab	16.9	<0.0001	12.8	48

Means (±SE) followed by the same letter in rows did not differ statistically from each other (Tukey test; $p > 0.05$).

[a] Days after insect removal from cages.

[b] Stink bug injury, rating scale 1–4 (Roza-Gomes et al., 2011), 0: no damage, 1: leaves with small punctuations, plant with no size reduction, 2: injured whorl (partially twisted), plant with size reduction, 3: twisted whorl or "suckering" plants (plants with tillers from the base), 4: dead whorl.

[c] Original means followed by statistics performed on $\sqrt{X+0.5}$ transformed data.

[d] Original means followed by mean separation performed on $\sqrt{X}$ transformed data.

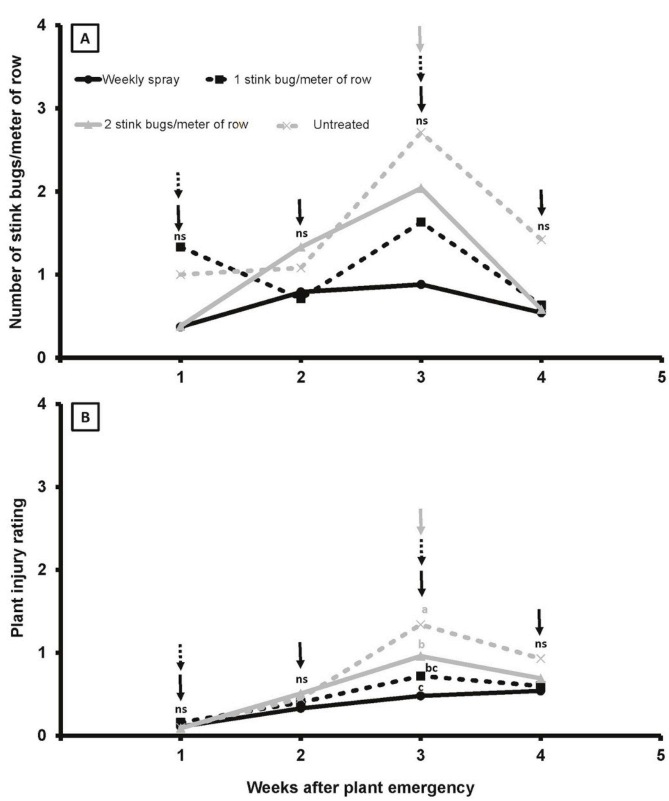

Fig. 5. Seasonal abundance of stink bugs, and plant injury rating in maize (BM 709 PRO2). The arrows indicate the timing of insecticide application in each treatment. Means followed by the same letters, in each evaluation date, did not statistically differ (Tukey test at 5% probability).[ns]ANOVA not significant.

technology, and (4) an ecological imbalance caused by the improper use of broad-spectrum insecticides, which are frequently overused (Bueno et al., 2011; Corrêa-Ferreira et al., 2010). Those factors will only worsen if the ET approach is not adopted or if the proposed ET is too low. Despite the economical importance of damage by stink bugs, our data shows that control by insecticides should be adopted with caution. We found that a higher ET (up to 3 adults of *D. melacanthus* m^{-1} of row) can safely be applied, and strongly recommend to avoid a reduced ET or "prophylactic" insecticide spraying without considering the pest population.

In summary, this study demonstrates that densities up to 3 adults of *D. melacanthus* m^{-1} of row do not reduce yield compared with the control. Although *E. heros* is present in maize crops in Brazil, its potential for damaging maize is low and a yield reduction or impact on the evaluated parameters was not evident in our study. Therefore, initiating a control at the studied densities (up to 6 stink bugs m^{-1} of row) is not justified. In addition, stink bug nymphs and adults might not trigger the same injuries, a finding that needs further research in order to refine current ETs in the future. Differences in injury caused by nymphs and adults as well as by different species should be examined.

Given the ability of maize to tolerate a stink bug infestation at an ET of 3 *D. melacanthus* adults per meter of row, it is crucial to apply IPM aimed at monitoring and sampling the pest in order to prevent inappropriate (abusive) use of insecticides. Stink bug sampling in maize is commonly performed based on visual counting what might bring some uncertainties surrounding this knowledge if not properly done. That is the reason sampling methods and other techniques are constantly revised to enhance their precision and practicality besides reducing costs (Onstad et al., 2019). Despite of this, good estimates of economic thresholds are needed (Nyrop et al., 1999) to solve common problems for IPM practitioners regarding to pest management decisions, that in a common field scenario, must be made rapidly and precisely to avoid economic loss and abusive use of pesticides (Onstad et al., 2019).

In addition, it must be emphasized that there may be differences in plant susceptibility to stink bug injury between different maize hybrids, underlining the importance of more studies in this subject. In general, the maize plant has small foliar plasticity, low prolificacy and low effective space compensation capacity (Pereira et al., 2012). Therefore, if stink bugs kill plants at emergence, plant stand losses cannot be effectively compensated by healthy plants in the surroundings, and new leaves will not develop to compensate damaged ones. These facts need to be taken into consideration in future research that will help to refine ETs

Table 2
Plant parameters at harvest of maize (BM 709 PRO2) following different economic thresholds (ETs) for stink bugs under field conditions (trial 4). Trial carried ou in Londrina, Paraná, Brazil on 2018.

Parameter	Insecticide spray timing				Statistics			
	Weekly spray	1 stink bug/meter	2 stink bugs/meter	Untreated	CV	*p*	F	df
Grain yield (kg.ha^{-1})	4479.2 ± 251.4^{ns}	4782.9 ± 265.2	4684.9 ± 464.4	4965.4 ± 359.5	10.6	0.5981	0.66	15
Net income (kg.ha^{-1})[a]	3499.2 ± 251.4 b	4292.9 ± 265.2 ab	4439.9 ± 464.4 ab	4965.4 ± 359.5 a	11.6	0.0165	5.90	15
Weight of 1000 grains (g)	248.5 ± 4.8^{ns}	258.7 ± 3.5	272.2 ± 12.6	260.2 ± 7.4	5.6	0.2218	1.77	15
Number of cobs/10 m	48.5 ± 0.5^{ns}	45.8 ± 2.1	45.5 ± 2.0	45.5 ± 0.7	6.6	0.4733	0.91	15
Flag leaf height (meter)	1.94 ± 0.03^{ns}	2.05 ± 0.04	1.96 ± 0.04	1.84 ± 0.06	5.3	0.1048	2.75	15
Main cob height (meter)	1.12 ± 0.04^{ns}	1.16 ± 0.04	1.15 ± 0.03	1.06 ± 0.04	7.4	0.3807	1.15	15

Means (±SE) followed by the same letter in rows did not differ statistically from each other (Tukey test; $p > 0.05$). ^{ns}ANOVA not significant.

[a] Net income calculated considering US$ 160.0/maize tonne in 2017 and insecticide cost of US$ 14.25/hectare/application + spraying cost of US$ 25.0/hectare/application (FAOstat, 2019).

for stink bugs in maize.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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